Reversed-phase method development typically includes screening and optimizing chromatographic parameters such as gradient slope, flow rate, temperature, column length, organic solvent and modifier concentration, and so on. The adjustment of pH can be a crucial variable in these screening tools. Relatively small changes in pH can significantly alter the selectivity between similar compounds and those with common functional groups. We have developed an efficient automation of such experiments by integrating the blending of stock buffers to the LC system to deliver mobile phases of different pH and ionic strength. To extend this utility to other compounds often requires selection of a different buffer system over a different pH range. It would be useful to have a protocol which can be applied to unknown compounds consisting of different functional groups. Such a general buffer system can then be used for different applications such as impurity profiling, stability testing and can be used with unknown compounds that have a mix of analyte sensitive to different ranges in the pH spectrum.

For this purpose, we have developed and validated a buffer system that provides buffering capacity over a wide pH range. Using this validated buffer system, we will demonstrate the effect of pH change on chromatographic properties of acidic and basic neutral compound mixtures. This systematic screening protocol using a single defined buffer system will prove to be useful for reversed-phase method development.

**PRINCIPLES OF AUTO-BLEND PLUS TECHNOLOGY**

AutoBlend Plus™ is a tool offered with the ACQUITY UPLC® H-Class Quaternary Solvent Pump. It is a combination of instrument characteristics and software algorithms with a user interface that lets us work in the units of pH and molarity. The user can directly run a pH gradient keeping the pH constant or run a pH as well as salt gradient. Based on well-understood principles, AutoBlend Plus™ calculates the proportion of solvents to deliver pH at 3.0 and 11.0. The plot of retention time against pH, shown in Figure 8, is an important method development tool, that helps decide the optimum pH for a separation. pH 3.6 is optimum in this example.

**METHODS**

**ACQUITY UPLC® H-Class with PDA Detector**

**LC System:** Acquity UPLC® H-Class with PDA Detector

**Column:** Acquity UPLC® 150 x 2.1 mm, 1.7µm

**Temperature:** 40°C

**Mobile Phase:**

- **Original Buffer System:**
  - Aqueous buffer (pH 10.86)
  - Line A: 100mM Phosphoric Acid in Water
  - Line B: 100% Sodium Phosphate Tribasic & Sodium Borate

- **Modified Buffer System:**
  - Line A: 200mM (Phosphoric and Citric acid) in Water
  - Line B: 100% Sodium Phosphate Tribasic & Sodium Borate in water
  - Line C: 100% Acetate (ACN)
  - Line D: 100% Water

**Separation Mode:** Gradient (5% to 50% Solvent C in 5 minutes)

**Flow Rate:** 0.6 ml/min

**Injection Volume:** 1.0 µL

**RESULTS AND DISCUSSION**

In reversed phase chromatography method development, an improper choice of buffer, in terms of buffering species, ionic strength & pH, can result in poor or irreproducible retention and failure in fixation of ionizable and polar compounds. Proper mobile phase buffering can overcome problems, such as, partial ionization & strong interaction between analytes and residual solvents or other active sites on the stationary phase. The blue curve below is the titration curve for the most common PA & SPT buffer system that spans a wide pH range of 2.3 to 11.0. To apply this buffer system to unknown compounds consisting of different functional groups thereby requiring buffering at different pH’s, we have calculated the optimum, specific and neutral pH in the above-mentioned pH range.

**CONCLUSION**

- **Reversed phase selectivity is sensitive to pH**
- **AutoBlend Plus™ facilitates systematic testing of pH**
- **Exploring pH is useful for methods development**
- **Systematic screening protocol with a single defined buffer system is useful for reversed phase method development.**
- **AutoBlend Plus™ facilitates systematic pH scouting and identifying a pH to develop a robust reverse phase method.**